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⑤④ Siliermittel

⑤⑦ Siliermittel, enthaltend eine wirksame Menge mindestens  
eines Hemmstoffes methanogener Bakterien.

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<p>(54) Title: ENSILAGE AGENT (54) Bezeichnung: SILIERMITTEL (57) Abstract An ensilage agent containing an active quantity of at least one methanogenic bacteria inhibitor. (57) Zusammenfassung Siliermittel, enthaltend eine wirksame Menge mindestens eines Hemmstoffes methanogener Bakterien.</p>		

## Ensiling composition

## Abstract

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Ensiling compositions contain an effective amount of at least one inhibitor of methanogenic bacteria.

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## Ensiling composition

The present invention relates to an ensiling composition containing an effective amount of at least one inhibitor of methanogenic bacteria. The present invention also relates to a process for the preparation of the ensiling composition and its use for ensiling green forage and preventing aerobic and anaerobic degradation processes in the silage.

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The production of silage is of extremely great commercial importance in animal nutrition. A number of additives and processes for improving the fermentation process and for improving the silage quality are known. Thus, the currently valid list of the ensiling compositions tested by the Deutsche Landwirtschaftliche Gesellschaft (DLG) describes the use of salts of formic acid and of propionic acid, nitrite compounds, sorbic acid, formaldehyde-generating compounds and sugar-containing substances, eg. molasses.

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DE-A 39 16 563 describes the addition of lactic acid-producing bacteria for improving the silage quality. The use of effective ensiling compositions reduces the feed losses due to nutrient degradation and prevents partial or complete spoilage through incorrect fermentation.

In general, in order to obtain a very good silage, it is necessary for a sufficient amount of lactic acid to be produced very rapidly and to be maintained in the silage for the stated storage period.

Important parameters for good ensiling are, for example, the buffer capacity of the forage plants, the contents of antimicrobial secondary ingredients in the plants, the acid- and osmosis-tolerance of the naturally occurring and of added lactic acid-producing bacteria, the metabolic efficiency of the natural epiphyte population, the presence of a sufficient amount of fermentable carbohydrates and anaerobic conditions in the feedstock.

There are close interactions between these parameters. In the presence of atmospheric oxygen, fermentable carbohydrates can be degraded by plant parts which are not yet dead or enzymes thereof and by aerobic microorganisms. This process involves a considerable nutrient loss, which inhibits development of lactic acid bacteria and at the same time makes it possible for silage pests to grow. This process continues until the atmospheric oxygen has been consumed. Under unfavorable conditions, this results in



failure to reach the rapid and necessary pH reduction in the material being ensiled. These are favorable growth conditions for Clostridia. Butyric acid bacteria (Clostridia) are anaerobic bacteria which, in the absence of oxygen, degrade organic substrates to alcohols, acids, CO<sub>2</sub> and N<sub>2</sub>. The acid formed is preferably butyric acid. The growth of the Clostridia and the associated formation of butyric acid considerably impair the quality of the silage. This butyric acid fermentation can lead to complete spoilage of the silage.

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Clostridia are known to spoil feeds and foods.

German Laid-Open Application DOS 2,613,503 discloses organic acids for ensiling green forage. Lactic acid bacteria for ensiling green forage and a combination of organic acids and lactic acid bacteria are described in EP 369 198 B1 and EP 399 385 B1, respectively.

It is possible to suppress Clostridia by adding nitrite. However, the addition of nitrite is problematic from the toxicological point of view.

In spite of this use of nitrite or nitrate in ensiling, silages suffer a constant weight and quality loss due to respiration of the sugars and starch by these undesirable microorganisms. This leads to considerable economic damage.

It is an object of the present invention to prevent the development of butyric acid-forming bacteria (Clostridia) in the silage, without the abovementioned disadvantages, and thus to suppress anaerobic degradation processes of the silage in a controlled manner.

We have found that this object is achieved and that, by adding inhibitors of methanogenic bacteria to the silage, the growth of the Clostridia can be suppressed in a controlled manner and the fermentation and quality losses of silages can thus be substantially reduced. The present invention relates to an ensiling composition containing an effective amount of at least one inhibitor of methanogenic bacteria.

The present invention also relates to a process for the preparation of the ensiling composition and its use for ensiling green forage and preventing aerobic and anaerobic degradation processes in the silage.





Inhibitors of methanogenic bacteria which are suitable for the novel ensiling composition are all substances which selectively inhibit the growth of methanogenic bacteria. Methanogenic bacteria are to be understood as meaning, for example, the following families and genera of Archaea: Methanobacteriaceae, Methanobacterium, Methanobrevibacter, Methanospaera; Methanotherma-  
ceae, Methanothermus; Methanococcaceae, Methanococcus; Methano-  
sarcinaceae, Halomethanococcus, Methanococcoides, Methanohalo-  
bium, Methanohalophilus, Methanolobus, Methanosarcina, Methano-  
saeta; Methanomicrobiaceae, Methanoculleus, Methanogenium, Metha-  
nolacinia, Methanomicrobium, Methanoplanus, Methanospirillum; Me-  
thanocorpusculaceae, Methanocorpusculum.

Inhibitors of methanogenic bacteria are, for example, antibiotics  
such as puromycin and/or bacitracin, bacteriocins and/or methano-  
genesis inhibitors, such as bromoethanesulfonic acid and/or  
monensin. Preferably used inhibitors are methanogenesis inhibi-  
tors, very particularly preferably bromoethanesulfonic acid and/  
or monensin.

Individual inhibitors or combinations of different inhibitors may  
be used.

The inhibitors may be used alone for ensiling green forage and  
preventing anaerobic degradation processes in silage or in com-  
bination with other ensiling agents, lactic acid-producing  
bacteria and/or assistants for ensiling green forage and prevent-  
ing aerobic and anaerobic degradation processes in silage.

The inhibitors of methanogenic bacteria are used in an amount of  
from 0.01 to 99.9, preferably from 0.05 to 90, particularly from  
0.5 to 70, % by weight, based on the total ensiling amount [sic]  
(= 100 % by weight).

The inhibitors of methanogenic bacteria can advantageously be  
used in an amount of from 0.001 to 10, in particular from 0.05 to  
8, very preferably from 0.1 to 5, kg, based on one metric ton of  
material being ensiled.

Suitable ensiling agents are all known inorganic and organic en-  
siling agents. Inorganic ensiling agents are, for example, cal-  
cium carbonate, magnesium carbonate, cobalt carbonate, sodium  
sulfate, ammonium sulfate, manganese sulfate, cobalt sulfate,  
iron sulfate, zinc sulfate and/or copper sulfate, sodium metabi-  
sulfite, orthophosphoric acid, sodium nitrate, sodium nitrite,  
sodium chloride, potassium chloride, calcium phosphate, calcium  
silicate, kieselguhr and mineral acids, such as hydrochloric acid



and/or sulfuric acid. Sodium nitrite, sodium metabisulfite, sodium chloride and/or potassium chloride are preferred.

Organic ensiling agents are to be understood as meaning, for example, formaldehyde, paraformaldehyde, hexamethylenetetramine, organic acids and/or agriculturally useful salts thereof, without being restricted to these. Aliphatic C<sub>1</sub>-C<sub>7</sub>-mono-, di- and tricarboxylic acids, benzoic acid and/or agriculturally useful salts thereof, for example formic acid, acetic acid, glycolic acid, propionic acid, lactic acid, valeric acid, caproic acid, heptanoic acid, acrylic acid, maleic acid, fumaric acid, citric acid and sorbic acid, are preferably used. Particularly preferred acids and/or agriculturally useful salts thereof are citric acid, formic acid and/or propionic acid. Particularly preferred salts are calcium formate, sodium formate, sodium propionate and/or calcium propionate.

Agriculturally useful salts of the abovementioned acids are salts of those bases which do not impair the ensiling effect.

Particularly suitable basic salts are those of the alkali metals, preferably the sodium and potassium salts, those of the alkaline earth metals, preferably calcium, magnesium and/or barium salts, and those of the transition metals, preferably manganese, copper, zinc, cobalt and/or iron salts, and the ammonium salts.

The ensiling agents can advantageously be used in an amount of from 0.1 to 99.99, preferably from 10 to 99.95, in particular from 25 to 99.5, % by weight, based on the total amount of ensiling composition (= 100 % by weight).

The ensiling agents may be added individually or in combination with the ensiling composition.

Strains of lactic acid-producing bacteria (LAB) which may be added to the novel ensiling composition are in principle all those which grow under the conditions prevailing in the silage and produce sufficient lactic acid, ie. form lactic acid in such an amount that the resulting decrease in pH is so rapid that no undesirable microorganisms can develop.

Suitable LAB are advantageously those of the genera Lactobacillus, Lactococcus, Streptococcus, Leuconostoc, Carnobacterium, Staphylococcus, Aerococcus and/or Pediococcus. Microorganisms of the genera Lactococcus, Lactobacillus and/or Leuconostoc are particularly suitable. The genus and species Lactobacillus plantarum is very particularly suitable. The bacteria may be used





in fluid, frozen or lyophilized form. Before use, they may be applied to a substrate and/or mixed with further assistants and/or granulated.

- 5 Examples of suitable substrates are calcium carbonate, bran, semolina, granulated sugar, bone feed meal, bole, talc, stearates of alkaline earth metals, sodium chloride, rock salt or other minerals suitable for feeding. When the bacteria are mixed with or applied to the substrate, it should be ensured that the bacteria  
10 are not damaged. Individual bacterial strains or combinations of different strains may be used.

The bacteria can advantageously be used in an amount of from 0 to 10, preferably from 0 to 5, in particular from 0 to 1, % by  
15 weight, based on the total amount of ensiling composition (= 100 % by weight).

- For the purposes of the present invention, assistants are substances which promote the growth of microorganisms, such as fermentable carbohydrate-containing products, for example molasses,  
20 boiled potatoes, cereal meal, dried whey, green spelt meal, dried sugar beet pulp, sugar, starch hydrolysis products or wood hydrolysis products.

- 25 The addition of cellulolytic, amylolytic and/or other carbohydrate-degrading enzymes or nitrogen in bound form, for example urea or ammonium compounds, such as ammonium sulfate, also has a growth-promoting effect.

- 30 Assistants may advantageously be added to the ensiling composition prepared according to the invention in an amount of from 0 to 60, preferably from 0 to 50, very particularly preferably from 0 to 30, % by weight, based on the total amount of ensiling composition (= 100 % by weight).

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- The silage which can be used according to the invention is not subject per se to any particular restriction. All readily ensilable feeds (slightly wilted grass and other feed plants containing more than 3 % of sugar in the fresh matter, wilted grass containing > 30 % of dry matter, wilted leguminosae containing  
40 > 35 % of dry matter, silo corn and corn cob products containing > 25 % of dry matter, whole-plant cereals, moist cereals and moist corn, pressed sugar beet pulp), feeds which are moderately difficult to ensile (grass containing from 20 to 25 % of dry matter, leguminosae containing from 25 to 30 % of dry matter and  
45 other feed plants having sugar contents of from 1.5 to 3 % in the fresh matter) or feeds which are difficult to ensile (grass con-



taining < 20 % of dry matter or leguminosae containing < 20 % of dry matter with less than 1.5 % of sugar in the fresh matter and grass or leguminosae which have been unsuccessfully wilted) are suitable. The material being ensiled preferably has a moisture content of at least 50 %, very preferably from 60 to 90 % (corresponding to 40-10 % of dry substance).

The novel ensiling composition is added to the material being ensiled in an amount of from 0.01 to 20, in particular from 0.05 to 10, very preferably from 0.1 to 5, kg per metric ton of material being ensiled. The ensiling composition may be provided to the farmer as individual components or as a prepared mixture. In this case, it is advantageous to use the components in a form such that the components do not mutually influence one another in an adverse manner. This can be achieved, for example, by micronization. It is added to the material to be ensiled advantageously in the form of a distributable mixture of the components. However, the components may also be added separately to the material being ensiled. The ensiling composition is applied either manually or by means of applicator or a metering apparatus, during chaff-cutting and/or during or after storage of the green forage.

The examples which follow illustrate the invention and are preferred embodiments of the invention.

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#### Example 1

Alfalfa containing 19 % of dry matter was ensiled, and 4 g of ensiling composition per kg of material being ensiled were added in all examples. Batch 1 (Table 1) is the control without the addition of ensiling compositions. Batch 2 shows the effect of a conventional ensiling composition comprising 50 % by weight of sodium propionate and 50 % by weight of sodium formate.

35 Table I: Ensiling of alfalfa

40	Batch	Weight loss in g/kg of material being ensiled
	1	16.2
	2	7.1
	3	3

45 Batch 3 shows the dry weight losses in the case of an ensiling composition comprising 45 % by weight of sodium propionate, 50 % by weight of sodium formate and 5 % by weight of bromoethanesul-



fonic acid as a methanogenesis inhibitor. Table 1 shows the mean values from three independent experiments in each case.

The use of methanogenesis inhibitors significantly reduces the weight loss of the material being ensiled.

#### Example 2

Mixed meadow grass containing 23 % of dry substance was ensiled. Batch 1 (control) in Table II shows a weight loss of 5.8 g.

A mixture of 50 % by weight of sodium propionate and 50 % by weight of sodium formate (batch 2) showed a loss of 3.0 g. The same mixture containing 5 % by weight of bromoethanesulfonic acid had a weight loss of only 2.0 g. Here too, the mean values of three independent experiments are shown.

Table II: Ensiling of mixed meadow grass

Batch	Weight loss in g/kg of material being ensiled
1	5.8
2	3.0
3	2.0



THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. An ensiling composition containing an effective amount of at least one methanogenesis inhibitor of methanogenic bacteria.
2. An ensiling composition as claimed in claim 1, wherein bromoethanesulfonic acid or monensin is used as the methanogenesis inhibitor.
3. An ensiling composition as claimed in claim 1 or 2, wherein an inorganic or organic ensiling agent is used as the ensiling agent.
4. An ensiling composition as claimed in any one of claims 1 to 3, wherein the ensiling agent used is an organic acid or an agriculturally usable salt thereof.
5. An ensiling composition as claimed in any one of claims 1 to 4, wherein the ensiling agent used is an aliphatic C<sub>1</sub>-C<sub>7</sub>-mono-, di- or tricarboxylic acid, benzoic acid or an agriculturally usable salt thereof.
6. An ensiling composition as claimed in any one of claims 1 to 5, wherein the lactic acid-producing bacteria used are those of the genera Lactobacillus, Lactococcus, Streptococcus, Leuconostoc, Carnobacterium, Staphylococcus, Aerococcus or Pediococcus.
7. A process for the preparation of an ensiling composition as claimed in any one of claims 1 to 6, wherein inhibitors of methanogenic bacteria, ensiling agents, lactic acid-producing bacteria and assistants are mixed.
8. A process for the preparation of an ensiling composition as claimed in claim 7, wherein the individual components are granulated before or after mixing.



9. A material being ensiled, containing an ensiling composition as claimed in any one of claims 1 to 6.
10. A material being ensiled, containing from 0.01 to 20 kg of an ensiling composition as claimed in claim 9 per metric ton of silage.
11. A method for preventing anaerobic degradation processes in green forage silages, wherein an effective amount of an ensiling composition as claimed in any one of claims 1 to 6 is added to the green forage plants.
12. A method for preventing anaerobic degradation processes in green forage silages as claimed in claim 11, wherein one or more inhibitors of methanogenic bacteria are added to the green forage plants in an amount of from 0.001 to 10 kg per metric ton of material being ensiled.
13. Use of an ensiling composition as claimed in any one of claims 1 to 6 for ensiling green forage and preventing aerobic and anaerobic degradation processes in silage.
14. Use of one or more methanogenesis inhibitors for ensiling green forage and preventing anaerobic degradation processes in silage.
15. An ensiling composition as claimed in claim 1 substantially as hereinbefore described with reference to the examples.
16. A process for the preparation for an ensiling composition as claimed in claim 7 substantially as hereinbefore described with reference to the examples.

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